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Antioxidant activity of acetone extract of *Naravelia zeylanica*

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Abstract
Naravelia zeylanica Linn., is a climbing tree. The current study was to integrate the antioxidant activity of the leaves of Acetone extract of *Naravelia zeylanica Linn.,* belonging to the family Rannunculaceae, a popular drug in traditional medicine. Phytochemical investigation of Acetone extract of *Naravelia zeylanica Linn.,* Showed the presence of Sugar, Carbohydrates and Flavanoids. Jerzy W. Jaroszewski et al reported the presence of three simple benzamides, 3, 4-methylenedioxybenzamide, 4-methoxybenzamide and 4-hydroxy-3-methoxybenzamide. In the Acetone extract of *Naravelia zeylanica Linn.,* shows Antioxidant activity by the method of Superoxide scavenging activity and Hydroxyl Radical Scavenging Activity. But it does not show any antioxidant activity in Nitric oxide Radical Scavenging activity.

Keywords: Acetone extract, *Naravelia zeylanica*, Antioxidant, Hydroxyl Radical Scavenging.

1. Introduction
Medicinal plants are the local heritage with global importance. The World is endowed with a rich wealth of medicinal plants. In the olden traditions local communities in every ecosystem from the Trans Himalayas down to the coastal plains have discovered the medicinal use of thousands of plants found locally in their ecosystem [1].
Naravelia zeylanica Linn., known as Vatakkoti in Malayalam. Belongs to the family Ranunculaceae. It is a scandent or climbing shrub with tuberous roots, wiry stem and strong tendrils; leaves 3-foliate, opposite, terminal leaflets modified into a 3-branched tendril, leaflets ovate-lanceolate, serrate or crenate, prominently nerved; flowers yellow, fragrant, in axillary and terminal panicles, sepals downy, petals linear-clavate, elongate; fruits aggregate of achenes, ending in twisted feathery tails. In 2005 Jerzy W. Jaroszewski *et al* reported the presence of three simple benzamides, 3, 4-methylenedioxybenzamide, 4-methoxybenzamide and 4-hydroxy-3-methoxybenzamide [2]. Hence the present investigation attempt to bring out antioxidant studies of the leaves of acetone extract of *Naravelia zeylanica* Linn [3, 4].

2. Taxonomy [5]

Kingdom	Plantae
Subkingdom	Viridiplantae
Phylum	Tracheophyta
Subphylum	Euphyllophytina
Class	Magnoliopsida
Subclass	Ranunculidae
Order	Ranunculales
Family	Ranunculaceae
Genus	Naravelia
Botanical name	Naravelia zeylanica

3. Material and Methods
3.1 Collection and Preparation of plant material
Fresh leaves of *Naravelia zeylanica* Linn was collected from Sreekandapuram, Taliparamba, Kannur during the month of March. The leaves were washed several times with water to remove soil and extraneous matters, the leaves were spread on trays and air dried for two weeks.

They were kept at room temperature so that all water were removed and dried for two weeks. The dried leaves were powdered and sieved through No.10 sieve and the coarse powder [8] was collected for extraction.

3.2 Removal of Chlorophyll

The powdered leaf was packed in a filter paper in the form of a thimble and was pre extracted with petroleum ether (40 – 60 ^{0}C) in a Soxhlet extractor to remove chlorophyll, waxy matter etc. The marc was air dried and used for the preparation of acetone extract.

3.3 Preparation of Acetone Extract

The method employed for the extraction was continuous hot extraction. Defatted powdered drugs were packed in filter paper to form a thimble. The thimble was loaded in a Soxhlet extractor and extracted with acetone, till the extract coming out of the body of extractor was clear and colourless. When all the powder was extracted, the extracts were combined and 80% of the solvent was recovered using a distilling unit. The remaining solvent was removed by evaporation [6].

4. Evaluation of Antioxidant Activity

4.1 Superoxide Scavenging Activity

i) Principle

Super oxide was generated by photo reduction of riboflavin and the amount was measured by the reduction of NBT. Scavenging activity was measured by inhibition of NBT reduction in the presence of test compound.

ii) Method

The reaction mixture contained 2650 µl of phosphate buffer, 100 µl NBT, 200 µl KCN, 50 µl riboflavin and different concentrations of ethanolic extract of the plant in a final volume of 3 ml. The tubes were illuminated by an incandescent lamp for 15 minutes. Optical density was measured at 530 nm before and after illumination. The percentage of inhibition of super oxide generation was evaluated by comparing the absorbance value of control and test [7].

$$\text{Percentage inhibition} = \frac{C - T}{C} \times 100$$

C = absorbance of control
T = absorbance of the test

5. Hydroxyl Radical Scavenging Activity

i) Principle

Hydroxyl radical scavenging was measured by studying the competition between deoxyribose and the test compound for hydroxyl radical generated from the Fe^{3+}/ ascorbate/ EDTA / H_20_2 system (Fenton reaction). Deoxyribose attacks the hydroxyl radical and eventually results in the formation of TBARS (thiobarbituric acid reactive substances), which is estimated spectrophotometrically [8].

ii) Method

The reaction mixture contained 500 µl buffer, 100 µl H_2O_2, 100 µl EDTA, 100 µl $FeCl_3$, 100 µl Ascorbic acid, 100 µl deoxy ribose and various concentration of extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37 ºC. Took 800 µl from the reaction mixture; added 200 µl SDS, 1.5 ml acetic acid and 1.5 ml TBA and kept in boiling water bath for 1 hour. Cooled and added 1 ml of distilled 11000 water and 5 ml of pyridine: butanol mixture. Shaken well, Centrifuged and absorbance of the supernatant was taken at 530 nm [9].

6. Result

S. No	Group	% inhibition
1	Control	-
2	100 µg	11.11±0.020
3	200 µg	14.11±0.079
4	400 µg	18.06±0.020
5	600 µg	34.21±0.058
6	800 µg	51.56±0.33
7	1000 µg	55.74±0.040

± Standard deviation, *P>0.01 Vs Standard

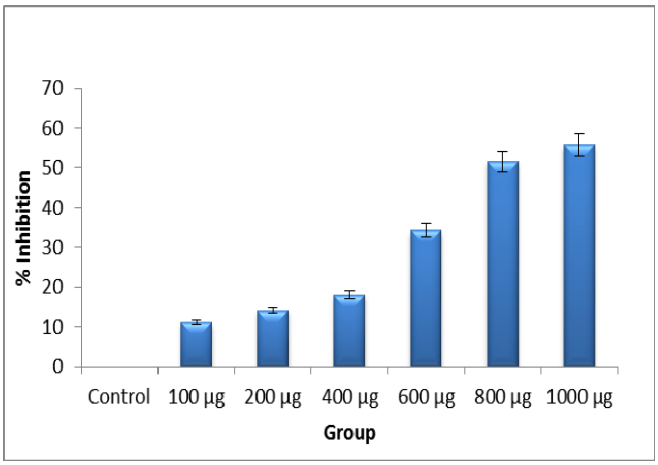

Fig 1: Effect of Extract on Superoxide Radical Scavenging Activity

SL. No	Group	% Inhibition
1	Control	-
2	100 µg	9.33±0.88
3	200 µg	18±0.14
4	400 µg	36±0.51
5	600 µg	45±0.66
6	800 µg	63±0.31
7	1000 µg	63±0.31

± Standard deviation, *P>0.01 Vs Standard

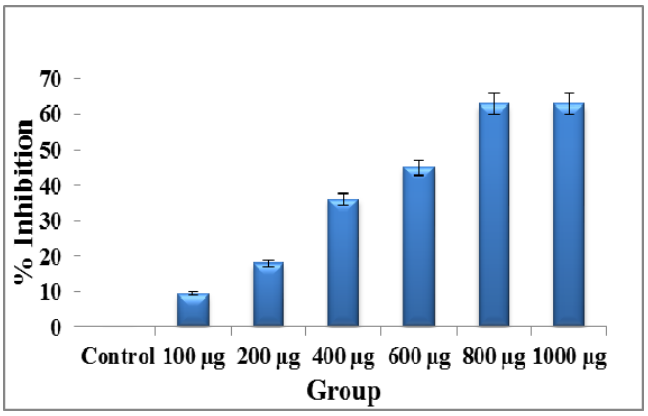

Fig 2: Effect of Extract on Hydroxyl Radical Scavenging Activity.

7. Discussion

The present study reveals that the acetone extract of the leaves of *Naravelia zeylanica* Linn., has significant antioxidant activity by Superoxide Scavenging Activity method and hydroxyl radical scavenging activity method.

Super oxide produced by the photo reduction of riboflavin was

found to be inhibited by acetone extract of *Naravelia zeylanica* Linn. The concentration of the leaf extract needed for 50% inhibition of super oxide was found to be 800 μg.

The hydroxyl radical generated by Fe^{3+}/ascorbate/H_20_2 system were inhibited by acetone extract of *Naravelia zeylanica* Linn). The concentration of the leaf extract needed for 50% inhibition of hydroxyl radical was found to be 800 μg Hydroxyl radical is highly reactive and short-lived.

Super oxide anion is the first reaction product of O_2 and it is a short lived species, generated in situ in normal cells under pathological conditions. In addition the metabolism of xenobiotics and exposure to ionizing radiations also generates these species. The most important source of O_2 radical is oxidative enzymes among which are xanthine oxidase, NADPH/NADH oxidase, aldehyde oxidase and dihydroorotate dehydrogenase etc. The condition of cellular oxidative stress arises either from over production of O_2 or other oxidative free radicals and results in tissue injury. Superoxide generated from activated neutrophils stimulates mutagenesis *in vitro* and oxidative stress from chronic inflammation favor cancer development in many organs.

The hydroxyl radical is produced following reaction of O_2 and H_2O in the presence of metallic ions such as Fe^{3+}/Cu^{+}. Lipid is very susceptible to OH radical attack and initiates lipid peroxidation. Also it induces conformational changes in DNA including strand breaks, base modification, damage to tumor suppressor gene and enhanced expression of proto-oncogenes

8. Conclusion

In conclusion, the present study revealed that *Naravelia zeylanica* Linn is a promising plant for future studies towards drug development and also the antioxidant activity of *Naravelia zeylanica* Linn., which indicates the need for the evaluation of anticancer and anti-inflammatory activities.

9. Reference

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